A new fluorescence imaging technique has enabled researchers to visualize initial step of the hematopoietic stem/progenitor cell (HSPC) homing. HSPC homing is an important biological phenomenon in which HSCs are transported from peripheral blood to bone marrow, providing an efficient treatment for blood disorders by bone marrow transplantation. Understanding detailed molecular mechanisms of this phenomenon is critical for the successful stem cell transplantation. However, nanoscopic spatiotemporal behavior of cellular interactions that regulate the HSPC homing has been elusive because of the limited spatial resolution and molecular specificity of the conventional experimental methods used in the relevant studies. A new microfluidics-based super-resolution (SR) fluorescence microscopy technique has enabled the capture and characterization of nanoscopic mechanism of cellular interactions mediated by ligand-receptor interactions occurring during an initial step in the HSPC homing.

Figure | Schematic illustration of the microfluidics-based super-resolution fluorescence imaging platform. The bottom panels show super-resolution images of selectin ligands captured before and after the cell rolling on the selectin surface.
Cellular interactions occurring in the presence of external forces play a key role in many biologically important processes (Nat. Rev. Immunol., 13, 159, 2013). In many cases, spatiotemporal dynamics of ligands and receptors on a cell surface governs cellular interactions as well as intracellular signaling. Although a wide variety of tools have been developed to characterize a diverse effect of external forces on biological processes (Nat. Rev. Mol. Cell. Biol., 15, 825, 2014), tools that can be used to visualize and characterize nanoscopic spatiotemporal behavior of ligand-receptor interactions in the presence of external forces are limited.

Habuchi et al. has developed a new fluorescence imaging technique that harnesses unprecedented spatial resolution of SR fluorescence microscopy technique in order to capture and characterize initial step of the HSPC homing (Nat. Rev. Immunol., 7, 678, 2007 | Annu. Rev. Cell Dev. Biol., 26, 363, 2010 | Mol. Immunol., 55, 59, 2013) mediated by the binding of E-selectin expressed on endothelium to selectin ligands expressed on HSPCs, which occurs in the presence of external shear force (Sci. Adv., 4, eaat5304, 2018). While SR fluorescence imaging techniques have become an essential tool for visualizing and characterizing subcellular structures and their dynamics (Science 313, 1642, 2006 | Science 319, 810, 2008 | Angew. Chem. Int. Ed., 47, 6172, 2008), SR imaging in the presence of external forces has remained a challenge. In their new microfluidics-based SR imaging platform, they mimic cellular interactions occurring in a blood stream by immobilizing endothelium molecules, selectin ligands, to the bottom of microfluidic chamber and flow a suspension of HSPCs into the chamber. The injected HSPCs are tethered to the surface of the chamber by to the selectin-ligand interactions and show rolling behavior on the surface due to the drag force exerted to the cells by the laminar flow. Nanoscopic spatial architectures of selectin ligands on the cells are then characterized by fixing the cells during rolling and visualizing their spatial distribution using the SR imaging.

The new imaging technique revealed that CD44, an E-selectin ligand clustered in a protruding structure out of the cell membrane of HSPCs, undergoes nanoscale reorganization of its clustering behavior, from patchy to elongated shape. The study demonstrated that the mechanical force exerted to the cell during the rolling by the selectin-ligand interactions is essential for this reorganization. This reorganization also caused a large structural reorganization of cortical actin cytoskeleton. The study also showed that this reorganization helps the HSPCs bind strongly to E-selectin that leads to slower and more stable rolling of the cell essential for efficient homing. Together, the new method revealed that the initial step of HSPC homing is far more dynamic and complicated than previously thought.

The new microfluidics-based SR imaging platform is applicable to investigate the subsequent steps in the HSPC homing and thus would be a powerful experimental platform for a broad studies in the field of HSPC homing. The method is, in principle, compatible with light-sheet microscopy that would expand the application of this technique to study HSPC homing using endothelium. We also foresee that the technique can be applied to a broader area of researches that target biological phenomena occurring in blood stream, including pathogen infection and cancer metastasis.

**Review Editor’s Views** || The author invented a flow-based super-resolution imaging system, possibly a technology of the future for Biology, Medicine and Health-care.